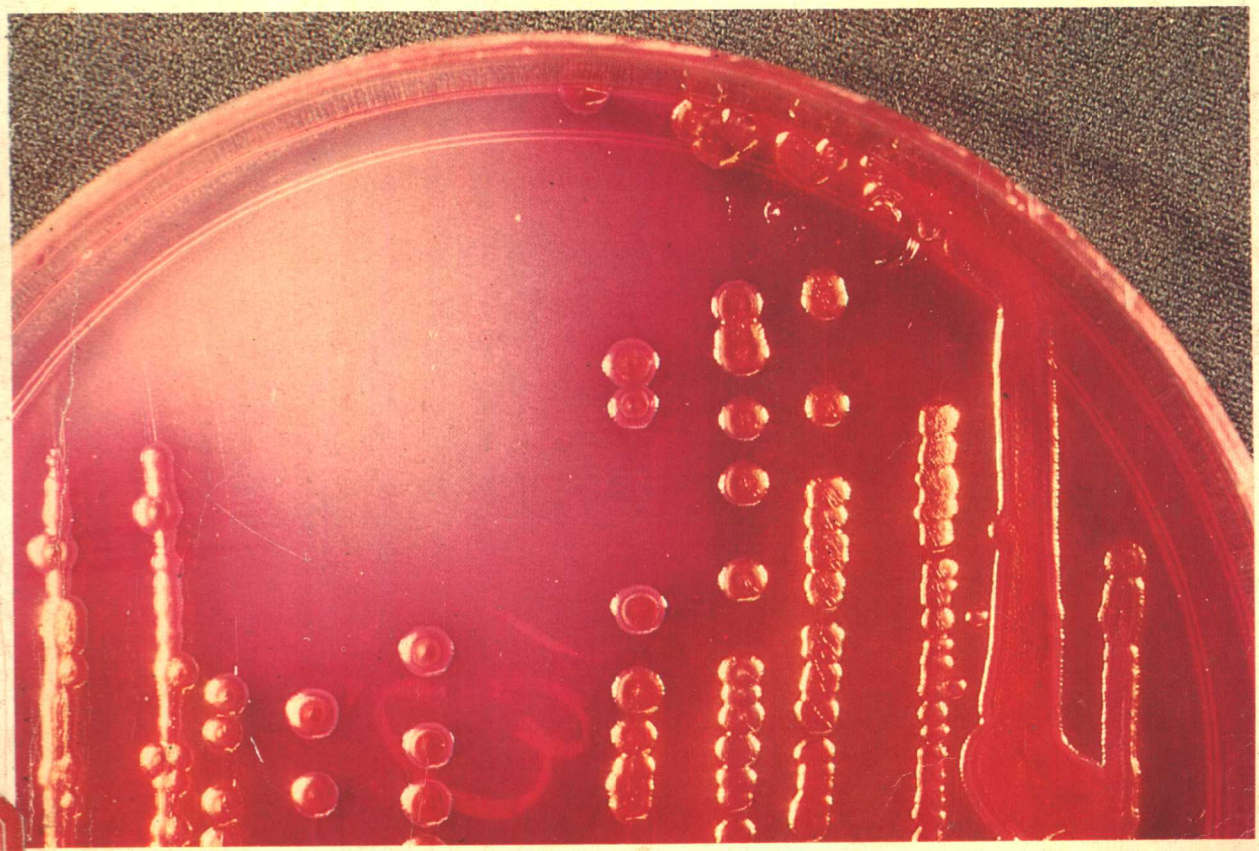
L. JACK BRADSHAW

LABORATORY MICROBIOLOGY

Third Edition



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front cover – Escherichia coli

back cover – *top:* Mycobacterium tuberculosis
bottom: Neisseria catarrhalis

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PREFACE
to the third edition

Since the last revision of this book, we have all witnessed a remarkable increase of student interest in the medical sciences. In many colleges and universities they constitute the overwhelming majority of biology majors. In keeping with this trend, I have enlarged the chapter dealing with clinical microbiology (formerly medical microbiology) so that a student can get a more coherent picture of that rapidly expanding field. Also included is an experiment on bacteriophage replication not found in previous editions.

Another innovation has been a major revision of the chapter on identification of unknown bacteria. Since the last revision there has been a new edition (8th) of *Bergey's Manual of Determinative Bacteriology*, and it was therefore necessary to bring all bacterial nomenclature in this book in line with that of the new *Manual*. A major problem surfaced, however, in that the new *Bergey's* did not attempt to itemize all known characteristics of each organism but used key differential tests only. In addition, many characteristics employed are not usually available to beginning students, for example, G-C ratios. I therefore attempted to follow my old format of using an introductory key, but then listed as many differential characteristics as could be found in both the 7th and 8th editions of *Bergey's Manual* for a given group of organisms in the hope that this would make determination of an unknown bacterium a feasible exercise for the beginning student. Many of my colleagues using the current edition of *Bergey's* have declared it confusing for their students, so I have tried to alleviate this problem. Since my students have found this portion of the course the most rewarding of all, I hope my purpose has been achieved.

The entire book has been carefully scrutinized for the need to update concepts and explanations. In addition, more liberal use of illustrations has been employed than in previous editions in the hope of greater clarity and usefulness. However, I have resisted the temptation to overuse illustrative material, feeling that reading comprehension still constitutes an important part of any college course.

I would like to thank Dr. John Lewis for his help and counsel in this and previous editions. Also my gratitude to Julie Knapp for her kind assistance, and to Judy Chambers, who typed the manuscript in this and previous editions. Finally, my thanks to Elaine Correia for her valuable suggestions and to the users of this book all over the country for their many suggestions.

L. JACK BRADSHAW

GENERAL MICROBIOLOGY LABORATORY INSTRUCTIONS

STUDENT EQUIPMENT

The following items of equipment should be available to each student:

One box of glass microscope slides (approximately 10).
One concave slide and one deep well slide.
One inoculating needle holder, one straight nichrome wire (4 in.) and one nichrome loop (3 in.)
One bent glass rod.
One slide holder.
Six coverslips.
A large handkerchief or small dish towel for drying slides.
Lens paper.
One tin can for boiling test tubes.

The following items should be available to students in the laboratory for general use:

Bensen burners.
Tripods and wire gauze to support cans of water for boiling.
Centigrade thermometers (0° - 100°).
Sterile Petri dishes.
Sterile pipets (1.0 and 10.0 ml. sizes).
Test tube racks or supports.
Bottles of sterile nutrient agar for plating (prescription bottles work well).
Incubator (37°C.).

GENERAL RULES OF LABORATORY CONDUCT

Although specific differences in rules exist from laboratory to laboratory, there are a few rules of conduct that are more or less universal in their applicability:

1. Eating or drinking is prohibited in the laboratory at all times.
2. Avoid putting any objects (such as pencils, fingers, etc.) in your mouth while working in the laboratory.
3. Wash your hands with soap and water at the conclusion of each laboratory period.
4. If a living culture of microorganisms is spilled, notify the instructor in charge of the laboratory immediately.
5. If you are injured in any way (burning is the most common type of injury sustained in this laboratory), notify the instructor immediately.
6. Make sure that all gas, water, and electrical appliances at your table are turned off before you leave.
7. Unless otherwise specified, all cultures are to be grown in the 37°C. incubator. If room temperature is called for, incubate in your drawer.
8. When melting a container of agar by boiling, make very sure that the cap is loosened slightly; otherwise the bottle may explode.
9. Be sure you wipe off the immersion oil before putting your microscope away.

CONTENTS

CHAPTER 1

INTRODUCTION TO THE MICROBIOLOGY LABORATORY

THE PURPOSE OF LABORATORY STUDY

Too many times the student views laboratory study as a tiresome assignment to be endured and the laboratory as a place where one only copies down results previously known. Because of this attitude, many students completely lose sight of the purpose of laboratory study. This is most unfortunate, because, given the proper attitude and a reasonable amount of intellectual curiosity, the student should find laboratory study one of the most fascinating parts of academic experience. An observer can usually tell when a good laboratory is in operation; the students are not anxious to leave and often spend many more than the scheduled hours in the laboratory. It is hoped that each student will be able to view the microbiology laboratory in this manner. Science, by its very nature, is a product of laboratory effort and thus one can say that real science is in the laboratory.

At this point, it might be well to call attention to a few simple but pertinent facts regarding the usefulness of laboratory study to the student. Undoubtedly, the main purpose of laboratory manipulation is to develop concepts learned from reading books and journals. In science, as in other fields of endeavor, there is often a large gap between book knowledge and applied knowledge. This is particularly true of modern microbiology, in which new and fascinating laboratory techniques and ideas are encountered almost daily in the journals. Unfortunately, many of these new techniques are predicated on the availability of excellent laboratory facilities to the experimenter. When the student is able to read about a certain phenomenon and then is able to follow this up by learning "how to do it" in a physical sense, learning is reinforced and a more realistic form of knowledge is acquired. It is quite true that many of the experiments set up in a student laboratory result in answers obvious to the experimenter before he ever begins, but it is by no means certain that the student can experimentally obtain this answer because of limited skill in laboratory techniques. You may know the result you're supposed to obtain, but you may not always be

able to get it when performing the experiment yourself. Often, even the most obvious experiment requires several repetitions before skill adequate to produce the desired results is acquired. Therefore, the "simple" experiment may become not so simple when viewed in this light.

A student who wishes to gain a full measure of return from his efforts in the laboratory should be prepared to repeat experiments that are not successful the first time. Satisfaction with a sloppy result only works to the detriment of the student. Perhaps it should be pointed out that there will be times when the desired results cannot be achieved even through repetition, simply because in dealing with living things the student is not working with unvarying entities. It is hoped that the experimental organisms provided are of the type designated, but often, particularly in microbiology, the student is plagued by mutative changes in these organisms, so that many times they no longer behave as originally anticipated. When this happens, it is possible for the experimenter to achieve the same incorrect result consistently despite repeated attempts. This often discourages students who hope to achieve ideal results (that is, predictable results). Herein lies one of the greatest challenges in biological experimentation – the student deals with fluid and pliable systems that usually remain predictable under specified conditions but are capable of unexpected variation. It is hoped that this idea will help to show the student that sometimes how he achieves the result is more important than the result itself. Careful attention to technique is essential.

A primary feature of the microbiology laboratory which tends to set it apart from many others is the "living" nature of virtually every experiment. Rarely will you be looking at preserved specimens or parts of organisms. The microorganisms employed are "alive and kicking," and as a result, certain rules of procedure must be adhered to very strictly. Although the vast majority of the microorganisms are harmless to you, some obviously are not. For this reason, you will be taught techniques which will apply to all microorganisms, whether or not they are disease-causing, so that your personal safety in the laboratory will be assured. If you develop an automatic or reflex attitude toward these basic techniques, you need have nothing to fear from any of your experimental subjects.

One of the prime goals of those who conduct a science laboratory is to give the student added incentive to study through interest developed in the laboratory. Most of us find it more interesting in the long run to observe the results of our own efforts rather than to read about the same results achieved by others. It is hoped that the experiments selected for this book have been set up to provide a maximum of understanding and interest. Let the student be assured that there is so much material that must be covered in the time allotted that there is no time whatsoever for work of the type that simply consumes time. Every experiment has been selected from many possible experiments that could have been used, and it is hoped that each one will teach the student something new and interesting.

One of the most important factors in laboratory study, from the student's point of view, is the attitude that he carries into the laboratory. If the student is interested in learning and is willing to put in both time and effort, he will find it one of the most pleasant and rewarding experiences of his

academic career. Carrying this attitude from the beginning, the student will find that the microbiology laboratory is an excellent stimulus to the imaginative mind. There are many experiments that could be performed that are not included in these laboratory instructions. If you want to perform experiments that are products of your own imagination, consult with the instructor, and the proper facilities probably will be made available.

REPORTING RESULTS

As in any experimental situation in which an objective is being sought, it is necessary to report the experiment in an organized fashion. Experience has shown that memory is an exceedingly poor repository for experimental observations; the only safe method is to write them down. There are many different ways of doing this, but ultimately the objective is always the same – to record the observations in an organized fashion and in the briefest possible manner. The experimental scientist ordinarily records results in a daily log sheet and then compiles these results into a publication or paper that condenses this record into a short but meaningful essay. In the laboratory you will report your results in the following form when appropriate (report sheets are provided in the book):

(a) *The statement of purpose* section should consist of one or two sentences that describe the principal purpose of the experiment to be performed.

(b) The *data* section should include no editorializing but simply a statement of results obtained from the experiment without further comment. Tables or graphs are frequently useful here; sometimes drawings are the only data.

(c) The *discussion and conclusions* section certainly should be the most meaningful part of the entire report. In this section you discuss the results obtained in light of the objectives with which you began. You will be expected to criticize the results you have obtained and the methods employed. This section will indicate whether or not you have fully understood the experiment. The final part of this section should be a numerical listing of the pertinent conclusions (if any) that you have reached as a result of your work. These conclusions should be brief, simple statements.

This, then, is the general pattern that is followed in most scientific publications; these reports will serve as practice in this technique. The results of the laboratory work will be kept by the student in a manner prescribed by his instructor. All records of results must be written in the laboratory so that they are in a legible and readable condition any time the instructor requests them. There will be periodic individual *unannounced* checks on your daily progress.

INTRODUCTION TO THE MICROSCOPE

One of the most useful tools of the microbiologist is the optical microscope. Although this instrument is used perhaps less than most people

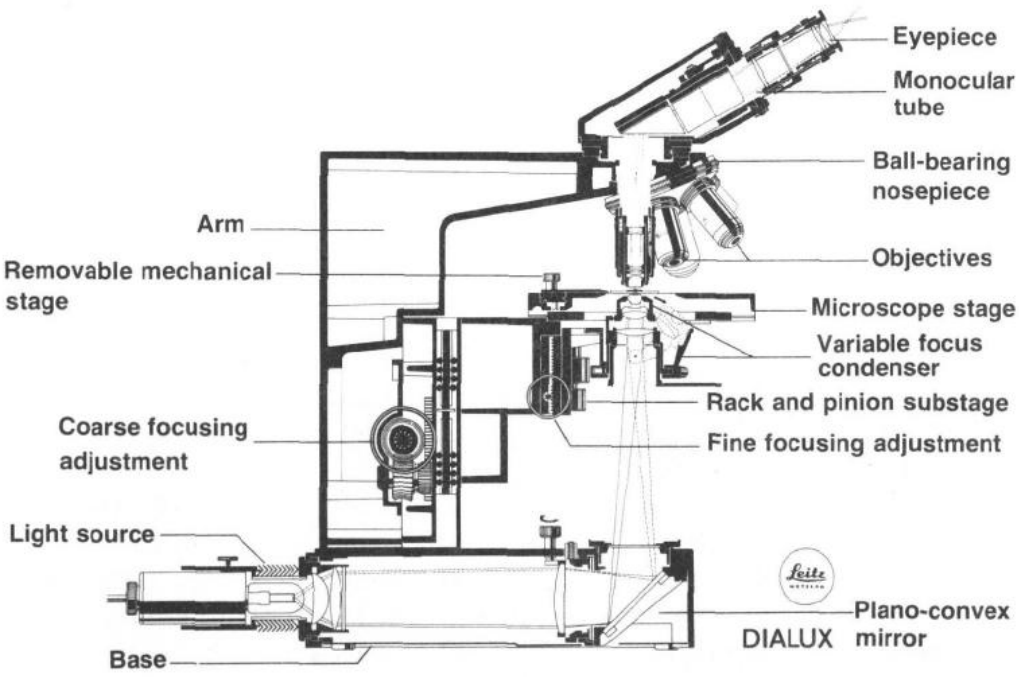

Figure 1-1 Typical working parts of the optical microscope. (Courtesy E. Leitz, Inc., Rockleigh, N.J.)

realize, it still is one of the most important instruments of study in microbiology and is certainly indispensable for certain types of work. All of the living forms with which you will be dealing are invisible to the naked eye, and a thorough familiarity with the microscope is essential at the very beginning of any laboratory course in microbiology. You will be expected to know all the major parts of the microscope and the function of each part.

Microscopes are considered either simple or compound, according to the number of lenses employed. Simple microscopes utilize single lenses, and compound microscopes utilize two or more lenses. Your microscopes are of the latter type, employing essentially two lens systems – the *ocular* or *eye piece*, and the *objective* lens. These two lenses are separated by a tube at such a distance that the eyepiece magnifies the image produced by the objective lens. In other words, the focal point of the objective lens is at some determined point within the tube, and the focal point of the ocular is set so that the ocular magnifies the image formed by the objective lens. The net result is a great enlargement of the object by the combined effects of the two lenses. Most microscopes used in microbiology require more than one type of objective lens since the magnifications needed differ from one type of experiment to another. Your microscopes are provided with three objective lenses, each giving a different magnification. The low power objective magnifies an object 10 times. The high-dry objective magnifies the object approximately 45 times, and the oil immersion objective magnifies an object 97 times. When combined with the ocular, which magnifies 10 times,

a practical magnification is achieved in each of the objective lenses of 100, 450, and 970 times, respectively. The term *magnification* means the enlargement of the linear diameter of an object.

Sheer magnification is not the only consideration the student is concerned with in the study of microorganisms. Undoubtedly the most important single theoretical consideration in the use of the microscope is a factor called *resolving power.* The resolving power of a microscope is a mathematical expression that denotes the ability of the lenses to distinguish detail clearly. One might say that the lens system loses its resolution of a given object when it can no longer separate two closely placed points or when the two points appear as one. Therefore, if a microscope cannot resolve an object, additional magnification is of no value, since it simply makes the blur larger. For all practical purposes, the limit of resolution of your microscope is approximately 0.2 μ.

A consideration of the details of operation of your microscope shows that operation of this instrument is based on the adjustment of several variables. You have two controls that move the optical system up and down, which serves to bring the combined lens system into proper focus. The *coarse adjustment* moves the tube rather rapidly, and the *fine adjustment* moves the tube very slowly. The coarse adjustment is capable of moving the *barrel* or *tube* of the microscope up and down throughout the entire length of the gears. The fine adjustment can only move through a limited number of turns, at which time it comes to a stop. Therefore, prior to use, the fine adjustment should be set in the *middle* of its course of travel so that you have a maximum of travel with it in either direction. Many microscopes are equipped with *mechanical stages.* These devices grip the microscope slide and enable very precise regulation of the movement of the slide. Sometimes it is necessary to observe without using the mechanical stage, in which case it can be very simply removed, as will be demonstrated by the instructor. Another variable concerns the amount of light entering the lens system, controlled largely by the *iris diaphragm.* When the diaphragm is closed down tightly, virtually no light is permitted to pass through; when it is fully open, it permits entry of a maximum amount of light. There is sometimes an adjustment on the *substage condenser,* which also has some influence on the amount and type of light entering. This will be discussed by the instructor. In general, most of your work should be done utilizing the maximum amount of light (iris diaphragm open wide).

A very important aspect of the operation of your microscope involves the proper use of the adjustment knobs in bringing the object to be studied into proper focus. Two of the objective lenses on your microscope can travel down onto the glass slide, which might result in damage to both lens and slide. It is, therefore, imperative that the student learn the proper method for focusing a microscope at the beginning. This method involves using the coarse adjustment to move the objective lens down as close to the slide as possible while watching from the side to make sure the slide and lens do not touch. *Never look through the eyepiece when turning the adjustment knobs downward.* When the lens is nearly touching the glass slide, then and only then look through the ocular and begin focusing *upward* until you come into

proper focus. The low power objective will not touch the glass when traveling to the bottom of its run, but both the high-dry and oil-immersion lenses will. Unfortunately, these are the most expensive of the objective lenses and are often subject to damage if pushed into the glass of the slide with any degree of force. Other details of the operation of your instrument will be given to you by your instructor.

Some of the common problems that beset beginning students while using the microscope should be mentioned at this time. There is an axiom in the field of electricity that states, "Always look for a blown fuse first in any electrical device that doesn't work." The lesson is simply that there are a few common recurring problems that generally constitute most of the difficulties in electricity or microscopy. Undoubtedly *the* most common problem is that of dirty lenses, both ocular and objective. All lenses should be cleaned with lens paper *each day* before use. The most effective method for cleaning lenses is the same as that employed for cleaning eye glasses. The student simply blows his breath on the lens to deposit a layer of condensation and wipes it off with the lens paper. Do not use handkerchiefs or other materials of this sort, since they usually make the lens dirtier. The eyepiece usually lifts out, and the "under" lens can be cleaned in the same way as the lens on the upper side. The objective lenses can ordinarily only be cleaned on the exposed surface, and in this case it is best done by taking a little tap water on your finger and moistening the lens surface, and then wiping it dry with lens paper. If this procedure still leaves the lens dirty, grease or oil may be the cause, in which case a small amount of xylene put on one corner of your lens paper and wiped briefly over the lens should remove the offending materials. Follow this with a brisk rubbing, using the dry part of your lens paper to remove the xylene. This solvent should be used quite sparingly, since in some microscopes the cement that holds the objective lenses in place is soluble in xylene, which could cause the lens to fall from its mounting. If you cannot see a subject through your microscope, the first thing you should look for is a dirty lens.

Another common error is the use of the high-dry lens instead of the oil-immersion lens. Since these two lenses are approximately the same length and look very similar, students sometimes use a high-dry lens with a drop of oil and wonder why the field of view is obscured. Make sure that you use the proper lens.

Yet another common mistake involves the proper centering of the lens to be used. The lens is not ready for use until it "clicks" into place under the barrel or tube of the microscope. Make sure that it is firmly snapped into place.

A common difficulty concerns improper illumination. On microscopes with fixed illumination, this does not present nearly so much a problem as it does with a mirror-type microscope. In general, however, your problems with illumination will be a matter of using too much illumination under certain circumstances, the field becoming so bright that you cannot see the object.

Special mention should be made regarding the cleaning of the oil immersion objective when you are through for the day. Wipe this lens with clean lens paper until no further evidence of oil is obtained. *Do not* use xylene or any other solvent in cleaning the oil from this lens.

Many microscopes are *parfocal* – that is, when one lens is in focus, the other two lenses will also be at the proper focal length if they are put into position without changing any adjustments. In practice this means that you may come into clear focus under low power (which is the easiest lens to bring into focus) and then, without moving anything else, by simply switching lenses to the one desired, you should be within one half turn (one way or another) with the fine adjustment of the proper focus.

EXPERIMENT 1-1

Introduction to the Use of the Microscope

MATERIALS

1. A strand of hair.
2. A letter "e."
3. Concentrated sodium chloride solution.
4. Gram-stained mixed culture of bacteria and yeasts.

PROCEDURE: Examine all materials listed above under the low power objective. It is easier to hold the hair securely under the microscope if you put it in the middle of a drop of water on a slide. This prevents it from blowing off the slide during the observation. The concentrated sodium chloride solution should be allowed to dry on the slide before observing. This causes the salt to form large crystals.

Repeat your observations using the high-dry objective. During observation with this objective, place a cover slip over your wet preparation to protect the lens from water. This is known as a wet mount. Make sketches under both low and high power of each different subject, except for the mixed culture slide. Observe this slide using your oil-immersion lens and draw a few representative organisms. Be sure to make your drawings definite or positive in outline. It is most important to pay attention to detail and actually to draw each subject in the field of view. Do not simply put down a mass of dots or lines from memory.